JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Conversion of Phenylalanine into Styrene by 2,4-Decadienal in Model Systems

FRANCISCO J. HIDALGO AND ROSARIO ZAMORA*

Instituto de la Grasa, Consejo Superior de Investigaciones Científicas, Avenida Padre García Tejero 4, 41012 Seville, Spain

2,4-Decadienal was heated under an inert atmosphere and in the presence of phenylalanine to investigate whether this secondary lipid oxidation product is a final product of lipid oxidation or it reacts with the amino acid. The results obtained showed that, in the presence of the alkadienal, the amino acid was degraded to styrene. This reaction was favored in dry systems at pH ∼ 6 and in the absence of oxygen. If oxygen was present, the alkadienal was oxidized and the Strecker degradation of the amino acid was produced. The activation energy for the formation of styrene from phenylalanine was 150.4 kJ/mol. The reaction mechanism is suggested to be produced either by an electronic rearrangement of the imine produced between the aldehyde and the amino acid with the formation of styrene, 2-pentylpyridine, carbon dioxide, and hydrogen, or by Michael addition of the amino compound to the alkadienal followed by β-elimination to produce the same compounds. Both reaction schemes were supported on the results obtained by studying both the degradation of phenylethylamine and phenylalanine methyl ester produced by 2,4-decadienal, and the formation of ethylbenzene in decadienal/phenylalanine reaction mixtures heated in the presence of platinum oxide. All these results suggest that, analogously to carbohydrates, certain lipid oxidation products may degrade appropriate amino acids to their corresponding vinylogous derivatives.

KEYWORDS: Acrylamide; alkadienals; carbonyl−amine reactions; flavors; lipid oxidation; Maillard reaction; Strecker aldehydes; styrene

INTRODUCTION

Amino acids are degraded by carbohydrates to the corresponding Strecker aldehydes. This reaction is one of the most important reactions leading to final aroma compounds in the Maillard reaction (*1*, *2*). In addition, recent studies have also shown that different lipid oxidation products are able to degrade amines and amino acids by a Strecker-type mechanism to the corresponding Strecker aldehydes and α-keto acids (*3*−*8*). This degradation is produced at 37 °C by lipid oxidation products having two oxygenated functions and needs stronger reaction conditions when the lipid oxidation product is an alkadienal or a ketodiene fatty ester.

The reaction pathway for the different lipid oxidation products assayed was always very similar, and the formation of an oxidized lipid with two oxygenated functions was needed to produce the amino acid degradation. Therefore, alkadienals and ketodienes had to be epoxidized to degrade the amino acid to its Strecker aldehyde (*8*). This oxidation is rapidly produced when the alkadienal or the ketodiene is heated in the presence of oxygen (*9*, *10*). However, in the absence of oxygen this oxidation is not produced and the amino acid is not degraded to the corresponding Strecker aldehyde (*8*).

The present investigation was undertaken to determine whether, under an inert atmosphere and in the presence of amino acids, alkadienals are final products of lipid oxidation or are able to modify amino acids to some extent. Model reactions were carried out using 2,4-decadienal, a major product of ω-6 fatty acid oxidation, and phenylalanine. In addition, other model systems involving 2,4-decadienal and phenylethylamine or phenylalanine methyl ester were also analyzed.

EXPERIMENTAL PROCEDURES

Materials. 2,4-Decadienal (93%), 2-ethylpyridine, L-phenylalanine, L-phenylalanine methyl ester, phenylethylamine, and styrene were obtained from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were purchased from reliable commercial sources.

Decadienal/Amino Compound Reaction Mixtures. Model reactions were carried out analogously to Granvogl and Schieberle (*11*). Briefly, mixtures of phenylalanine (phenylethylamine or phenylalanine methyl ester) and 2,4-decadienal (75 μmol each) were singly homogenized with 0.063−0.200 mm silica gel 60 (600 mg) (Macherey-Nagel, Düren, Germany) and 0−480 μL of 0.3 M buffer (sodium citrate for pH 3−6 and sodium phosphate for pH 6−7). In addition, some experiments also included catalytic amounts of platinum oxide, which was added before homogenization. Samples were heated under a controlled atmosphere at 180 °C in closed test tubes for 60 min, unless otherwise indicated. The oxygen content in the test tube was determined with a PAK01P Abiss analyzer (Viry-Chantillon, France). After cooling

* To whom correspondence should be addressed. Phone: +(34) 954 611 550. Fax: +(34) 954 616 790. E-mail: rzamora@ig.csic.es.

10.1021/jf070527w CCC: $37.00
Published on Web 05/09/2007

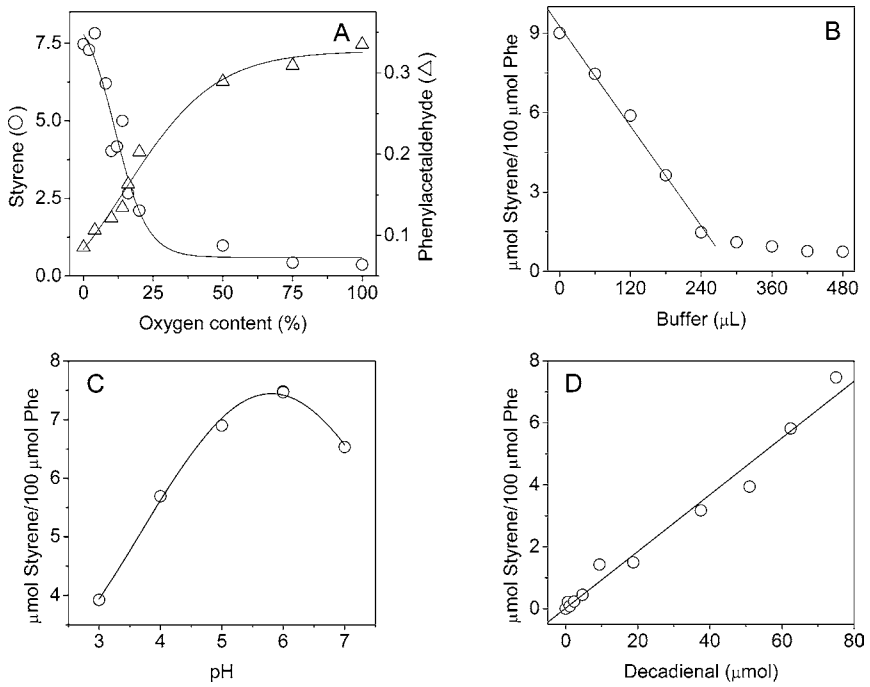

Figure 1. (**A**) Effect of oxygen content in the atmosphere of 2,4-decadienal/phenylalanine reaction mixtures on the formation of styrene (○) and phenylacetaldehyde (△). (**B**) Effect of the amount of buffer added on the styrene formation in 2,4-decadienal/phenylalanine reaction mixtures. (**C**) Effect of buffer pH on the styrene formation in 2,4-decadienal/phenylalanine reaction mixtures. (**D**) Effect of alkadienal concentration on the styrene formation in 2,4-decadienal/phenylalanine reaction mixtures. All results are given in μmol of compound produced/100 μmol of phenylalanine (Phe).

(15 min at −20 °C), 20−500 μL of water (the total volume of water plus buffer was 500 μL), 1 mL of acetonitrile, and 20 μL of internal standard solution (50 μL of 2-ethylpyridine in 10 mL of methanol) were added. Suspensions were stirred for 1 min, and the supernatant was filtered and studied by GC−MS.

GC−MS Analyses. GC−MS analyses were conducted with a Hewlett-Packerd 6890 GC Plus coupled with an Agilent 5973 mass selective detector-quadrupole type (MSD). A 30 m × 0.25 mm i.d. × 0.25 μm HP5-MS capillary column was used. Working conditions were as follows: carrier gas, helium (1 mL/min at constant flow); injector, 250 °C; oven temperature, from 70 (1 min) to 240 °C at 5 °C/min and then to 325 °C at 10 °C/min; transfer line to MSD, 280 °C; and ionization EI, 70 eV.

Determination of Styrene Contents. Quantification of styrene was carried out by preparing standard curves of this compound in the 1.52 mL of solution prepared for GC−MS injection. For each curve, 12 different concentration levels of styrene (0−75 μmol) were used. Styrene content was directly proportional to the styrene/internal standard area ratio ($r = 0.9999$, $p < 0.0001$). The coefficients of variation at the different concentrations were lower than 10%.

RESULTS AND DISCUSSION

Styrene Formation in Phenylalanine/2,4-Decadienal Reaction Mixtures. When phenylalanine was heated in the presence of 2,4-decadienal and in the absence of oxygen, the Strecker degradation of the amino acid was not produced. Nevertheless, the amino acid was not stable under the assayed conditions, and it was converted into styrene to some extent. Styrene could easily be identified by GC−MS on the basis of its retention index and mass spectra, and was the major reaction product in most experiments.

Styrene formation depended on the oxygen content in the atmosphere of the reaction mixture. **Figure 1A** shows that the amount of the produced styrene decreased rapidly (from ∼7.5 μmol/100 μmol of phenylalanine to ∼2 μmol/100 μmol of phenylalanine) and linearly ($r = -0.937$, $p = 0.0002$) when the amount of oxygen increased in the range 0−20%. Further increases of oxygen content also decreased the produced styrene, and in the presence of 100% oxygen, only 0.4 μmol of styrene/100 μmol of phenylalanine was formed.

In parallel to the decrease in the amount of the formed styrene, an increase in the amount of oxygen should produce the oxidation of the alkadienal and, therefore, the Strecker degradation of the amino acid (*8*). As observed in **Figure 1A** the amount of phenylacetaldehyde produced increased linearly ($r = 0.977$, $p = 0.00015$) when the oxygen content of the atmosphere increased from 0% to 50%. In fact, the amount of the produced styrene and phenylacetaldehyde were inversely correlated ($r = -0.907$, $p = 0.0007$). Nevertheless, the yield of phenylacetaldehyde was lower than the yield obtained for styrene. This was likely a consequence of other reactions that also took place in the presence of oxygen. Particularly, the degradation of the amino acid to benzaldehyde was the main reaction produced at high oxygen contents.

In addition to the presence of oxygen, the amount of buffer added to the reaction mixture also determined the amount of styrene produced. As shown in **Figure 1B**, the amount of styrene decreased linearly ($r = 0.996$, $p = 0.0003$) when 0−240 μL of buffer was added to the reaction mixture. Addition of buffer amounts higher than 240 μL only produced slight decreases in the styrene produced. Thus, the phenylalanine/decadienal reaction mixture produced 1.48 μmol of styrene/100 μmol of phenylalanine when 240 μL of buffer was added, and styrene content decreased to 0.75 μmol of styrene/100 μmol of phenylalanine when 480 μL of buffer was added.

The pH of the added buffer also influenced the amount of styrene produced. **Figure 1C** shows the effect of buffer pH in the amount of styrene produced. Only pH values of 3−7 could be studied because these experiments were carried out with 60 μL of buffer and only buffers having these pH values maintained the reaction pH after heating. In addition, there was not any difference in the amount of styrene produced at pH 6 when either sodium citrate or sodium phosphate buffer was used. The

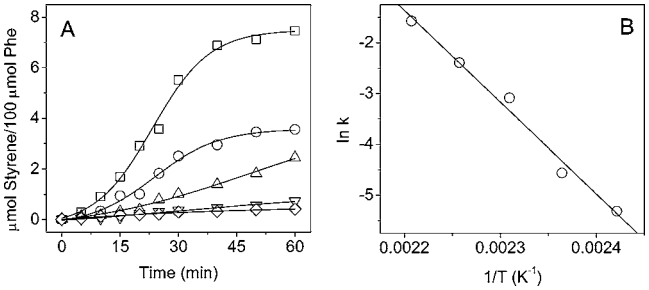

Figure 2. (**A**) Time courses of styrene formation in 2,4-decadienal/phenylalanine reaction mixtures heated at 140 (◇), 150 (▽), 160 (△), 170 (○), or 180 °C (□). (**B**) Arrhenius plot for styrene formation in 2,4-decadienal/phenylalanine reaction mixtures.

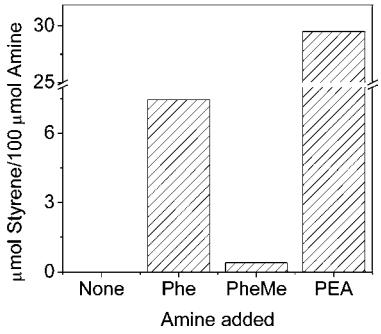

Figure 3. Effect of the amino compound added on styrene formation in 2,4-decadienal/amine reaction mixtures. Abbreviations: Phe, phenylalanine; PheMe, phenylalanine methyl ester; and PEA, phenylethylamine.

amount of styrene produced as a function of pH could be described using a Gaussian fit ($r^2 = 0.997$) with a maximum at pH ~ 6.

According to the results obtained and in order to maximize the amount of the produced styrene and to maintain the pH, the reaction conditions selected for this study were 100% nitrogen in the atmosphere and 60 μL of 0.3 M sodium phosphate, pH 6.

Effect of Phenylalanine/2,4-Decadienal Ratio in the Formation of Styrene. The alkadienal was responsible for the degradation of the amino acid because styrene was not produced in the absence of 2,4-decadienal (**Figure 1D**). In addition, the amount of produced styrene increased linearly ($r = 0.990$, $p < 0.0001$) with the amount of decadienal added. Under the assayed conditions, and using equimolecular amounts of decadienal and phenylalanine, the 7.5% of the amino acid was converted into styrene. As shown in **Figure 1B**, this yield increased to 9% in the absence of buffer.

Effect of Incubation Time and Temperature in the Amount of Styrene Produced in Phenylalanine/2,4-Decadienal Reaction Mixtures. The reaction rate and the amount of styrene produced depended on the incubation time and temperature (**Figure 2A**). Thus, the amount of styrene produced increased linearly ($r > 0.98$, $p < 0.0001$) with the incubation time at the lowest assayed temperatures (140−160 °C). At these temperatures the reaction mixtures were studied for 120 min (only the data obtained for the first 60 min are shown). At higher temperatures (170−180 °C) an initial lag lime was observed, and the styrene that formed achieved the maximum value after 30−40 min. The initial lag time was likely a consequence of the time needed for the system to achieve the reaction temperature. Therefore, after this lag time, styrene formation also increased linearly ($r > 0.98$, $p < 0.0001$) as a function of incubation time. After 1 h heating, the yield of styrene formation was 0.3% at 140 °C, 0.4% at 150 °C, 2.5% at 160 °C, 3.6% at 170 °C, and 7.5% at 180 °C.

Reaction rates at the different assayed temperatures were calculated using the following equation

$$[\text{styrene}] = [\text{styrene}]_0 + kt$$

where $[\text{styrene}]_0$ represents the intercept, k is the rate constant, and t is the time. These rate constants were used in an Arrhenius plot (**Figure 2B**) for calculation of activation energy (E_a) of styrene formation from phenylalanine in the presence of 2,4-decadienal. The value obtained for E_a was 150.4 kJ/mol.

Reaction Mechanism for the Formation of Styrene from Phenylalanine Produced by 2,4-Decadienal. In an attempt to determine the mechanism of degradation of phenylalanine, additional experiments were also carried out. In the first place, the reaction between phenylethylamine and 2,4-decadienal was studied to find out the role of the carboxylic group of the amino acid in this reaction. **Figure 3** shows that the absence of the carboxylic group did not inhibit the reaction. In fact, styrene was produced to a much higher extent in the degradation of the phenylethylamine than in the degradation of phenylalanine. Thus, after heating 1 h at 180 °C and in the presence of 60 μL of 0.3 M sodium phosphate, pH 6, the 30% of phenylethylamine was converted into styrene, and this yield decreased to 7.5% for the formation of styrene from phenylalanine.

The reaction of phenylalanine methyl ester with 2,4-decadienal was also studied. As expected, only very small amounts of styrene were detected (**Figure 3**). However, the formation of methyl cinnamate was not observed, therefore confirming that the presence of an ester group inhibited the reaction.

According to the obtained results, two reaction pathways may be proposed (**Figures 4** and **5**). In both cases, amino acid decarboxylation is produced as a first step, and the phenylethylamine formed may react with the alkadienal by two alternative pathways. In **Figure 4**, the reaction would be initiated with the formation of the corresponding imine that, at high temperature, would be fractionated as a consequence of an electronic rearrangement to produce styrene, 2-pentylpyridine, and hydrogen. In addition to styrene, 2-pentylpyridine was identified in the chromatogram on the basis of its retention index and mass spectra. To confirm that hydrogen was also produced, the reactions between phenylethylamine (or phenylalanine) and 2,4-decadienal were also carried out in the presence of platinum oxide. In the presence of this catalyst, the main compound produced in both reactions was not styrene but ethylbenzene, which was unambiguously identified on the basis of its retention index and mass spectra.

The alternative mechanism indicated in **Figure 5** is initiated by a Michael addition of the amine to the alkadienal followed by a β-elimination to produce styrene and a dihydropyridine which requires oxidation to produce the side product 2-pentylpyridine. As indicated in both schemes, both pathways are in agreement with the increased yield obtained for phenylethylamine and points out the difficulty of phenylethylamine methyl ester to produce the reaction. In addition, they explain the formation of the different reaction products and the production of ethylbenzene in the presence of platinum oxide. Furthermore, an increased water activity will favor neither the formation of the imine in **Figure 4** nor the production of the dihydropyridine in **Figure 5**.

Autoxidation of fats is the main reaction involved in the oxidative degradation of lipids, although lipids can be oxidized

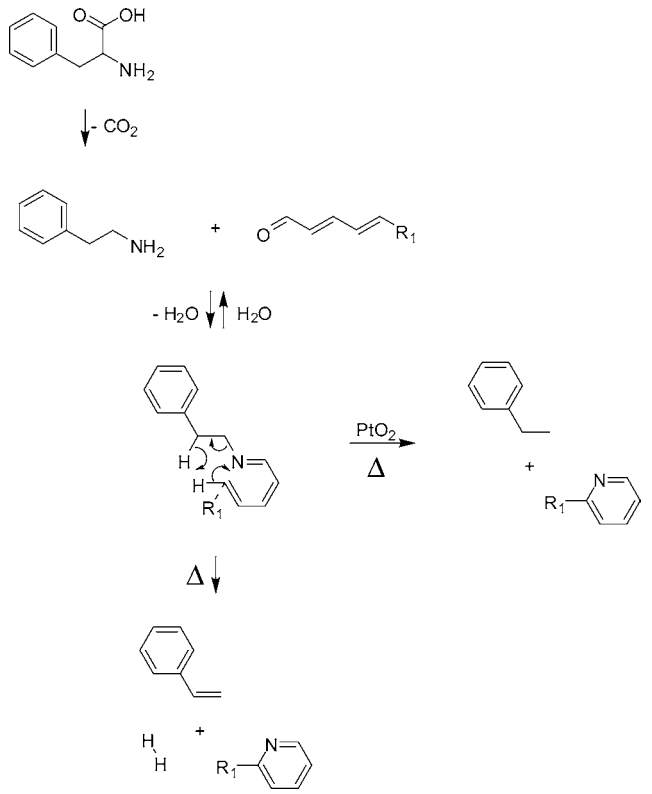

Figure 4. Reaction of 2,4-decadienal with phenylalanine or phenylethylamine via the formation of an initial imine. $R_1 = CH_3(CH_2)_4$.

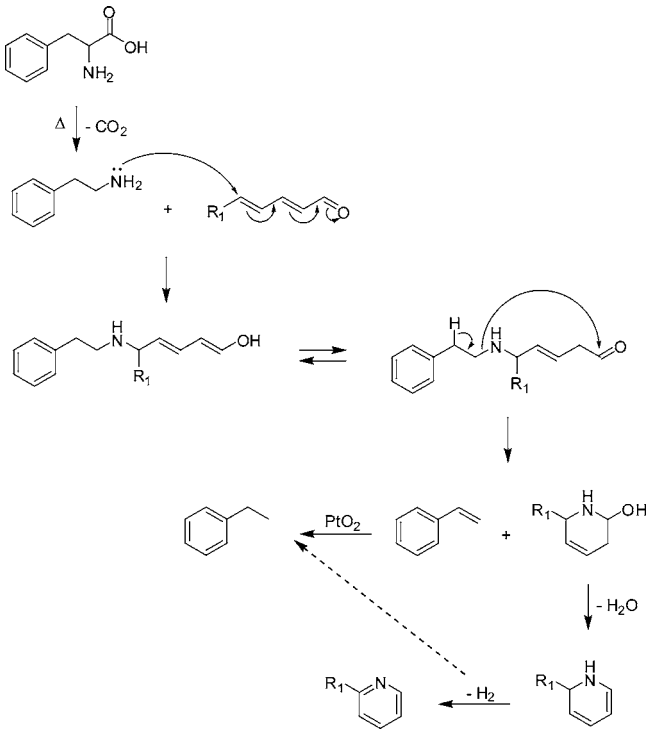

Figure 5. Reaction of 2,4-decadienal with phenylalanine or phenylethylamine initiated by the Michael addition of the amine to the alkadienal. $R_1 = CH_3(CH_2)_4$.

by both enzymatic and nonenzymatic mechanisms. The initial products of this oxidation are the corresponding hydroperoxides. These compounds are relatively unstable and enter into numerous complex reactions involving substrate degradation and interaction, resulting in a myriad of compounds of various molecular weights, flavors thresholds, and biological significance (*10*, *12*−*15*). Some of these reactions have long been known and are nowadays well characterized. On the other hand, many others are still either poorly characterized or just unknown.

This study has identified a new degradation pathway of amino acids produced by oxidized lipids, and suggests that alkadienals may not only be involved in the Strecker degradation of amino acids (*8*) but also in the degradation of amino acids to produce their corresponding vinylogous derivatives. This reaction is favored at pH ∼ 6 in dry systems. In addition, the presence of oxygen plays a major role in the reaction. Thus, the reaction between alkadienals and amino acids may follow two alternative mechanisms. If oxygen is present, the alkadienal is oxidized and the Strecker degradation of the amino acid is produced. In the absence of oxygen, the major product is the vinylogous derivative of the amino acid. Nevertheless, the activation energy of this last reaction is relatively high, and it is only expected to be produced to a great extent in systems submitted to relatively high temperatures. In addition, it is also expected to be amino acid specific and will depend on the amino acid structure to facilitate either the electronic shifts proposed in **Figure 4** or the β-elimination suggested in **Figure 5**.

Under the same reaction conditions, the reaction was favored when phenylethylamine was employed in the place of phenylalanine. Thus, the 30% of the amine was converted into styrene and only the 7.5% of phenylalanine was converted into this vinylogous derivative, therefore suggesting that the presence of the carboxylic group did not favor the reaction. When the carboxylic group was esterified, such as in phenylalanine methyl ester, styrene was detected in very small amounts and methyl cinnamate was not produced.

Previous studies have shown that the degradation of amino acids to their vinylogous derivatives is also produced in the Maillard reaction (*16*), and it is supposed to be responsible, at least in part, for the formation of acrylamide in thermally processed foods (*17*−*19*). Therefore, under appropriate conditions, oxidized lipids might also produce the degradation of asparagine to acrylamide, and contribute to the formation of this toxin during food processing. Additional studies are needed to confirm the participation of oxidized lipids in the formation of acrylamide. These studies are being carried out at present in this laboratory.

The results obtained in this and in previous studies suggest that a significant number of lipid oxidation products, some of which are quantitatively important, can produce the degradation of amino acids, analogously to carbohydrates. Therefore, both oxidized lipids and carbohydrates should be considered simultaneously to understand the changes produced in complex systems involving carbohydrates, lipids, and amino acids (*20*).

ACKNOWLEDGMENT

We are indebted to José L. Navarro for technical assistance.

LITERATURE CITED

(1) Whitfield, F. B. Volatiles from interactions of Maillard reactions and lipids. *Crit. Rev. Food Sci. Nutr.* **1992**, *31*, 1−58.

(2) Yaylayan, V. A. Recent advances in the chemistry of Strecker degradation and Amadori rearrangement: Implications to aroma and color formation. *Food Sci. Technol. Res.* **2003**, *9*, 1−6.

(3) Hidalgo, F. J.; Zamora, R. Strecker-type degradation produced by the lipid oxidation products 4,5-epoxy-2-alkenals. *J. Agric. Food Chem.* **2004**, *52*, 7126−7131.

(4) Zamora, R.; Gallardo, E.; Navarro, J. L.; Hidalgo, F. J. Strecker-type degradation of phenylalanine by methyl 9,10-epoxy-13-oxo-11-octadecenoate and methyl 12,13-epoxy-9-oxo-11-octadecenoate. *J. Agric. Food Chem.* **2005**, *53*, 4583−4588.

(5) Hidalgo, F. J.; Gallardo, E.; Zamora, R. Strecker type degradation of phenylalanine by 4-hydroxy-2-nonenal in model systems. *J. Agric. Food Chem.* **2005**, *53*, 10254−10259.
(6) Zamora, R.; Gallardo, E.; Hidalgo, F. J. Amine degradation by 4,5-epoxy-2-decenal in model systems. *J. Agric. Food Chem.* **2006**, *54*, 2398−2404.
(7) Zamora, R.; Navarro, J. L.; Gallardo, E.; Hidalgo, F. J. Chemical conversion of α-amino acids into α-keto acids by 4,5-epoxy-2-decenal. *J. Agric. Food Chem.* **2006**, *54*, 6101−6105.
(8) Zamora, R.; Gallardo, E.; Hidalgo, F. J. Strecker degradation initiated by 2,4-decadienal or methyl 13-oxooctadeca-9,11-dienoate in model systems. *J. Agric. Food Chem.* **2007**, *55*, 1308−1314.
(9) Gassenmeier, K.; Schieberle, P. Formation of the intense flavor compound *trans*-4,5-epoxy-(*E*)-2-decenal in thermally treated fats. *J. Am. Oil Chem. Soc.* **1994**, *71*, 1315−1319.
(10) Frankel, E. N. *Lipid Oxidation*, 2nd ed.; The Oily Press: Bridwater, U.K., 2005.
(11) Granvogl, M.; Schieberle, P. Thermally generated 3-aminopropionamide as a transient intermediate in the formation of acrylamide. *J. Agric. Food Chem.* **2006**, *54*, 5933−5938.
(12) Kamal-Eldin, A.; Pokorny, J. *Analysis of Lipid Oxidation*; AOCS Press: Champaign, IL, 2005.
(13) Steele, R. *Understanding and Measuring the Shelf-life of Food*; Woodhead Publishing Ltd.: Cambridge, U.K., 2004.
(14) Kamal-Eldin, A. *Lipid Oxidation Pathways*; AOCS Press: Champaign, IL, 2003.
(15) Zamora, R.; Alaiz, M.; Hidalgo, F. J. Determination of ϵ-*N*-pyrrolylnorleucine in fresh food products. *J. Agric. Food Chem.* **1999**, *47*, 1942−1947.
(16) Stadler, R. H.; Robert, F.; Riediker, S.; Varga, N.; Davidek, T.; Devaud, S.; Goldmann, T.; Hau, J.; Blank, I. In-depth mechanistic study on the formation of acrylamide and other vinylogous compounds by the Maillard reaction. *J. Agric. Food Chem.* **2004**, *52*, 5550−5558.
(17) Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Törnqvist, M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J. Agric. Food Chem.* **2002**, *50*, 4998−5006.
(18) Taeymans, D.; Wood, J.; Ashby, P.; Blank, I.; Studer, A.; Stadler, R. H.; Gonde, P.; Van Eijck, P.; Lalljie, S.; Lingnert, H.; Lindblom, M.; Matissek, R.; Muller, D.; Tallmadge, D.; O'Brien, J.; Thompson, S.; Silvani, D.; Whitmore, T. A review of acrylamide: an industry perspective on research, analysis, formation and control. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 323−347.
(19) *Chemistry and Safety of Acrylamide in Food*; Friedman, M., Mottram, D., Eds.; Springer: New York, 2005.
(20) Zamora, R.; Hidalgo, F. J. Coordinate contribution of lipid oxidation and Maillard reaction to the nonenzymatic food browning. *Crit. Rev. Food Sci. Nutr.* **2005**, *45*, 49−59.

Received for review February 22, 2007. Revised manuscript received March 30, 2007. Accepted April 2, 2007. This study was supported in part by the European Union (FEDER funds) and the Plan Nacional de I + D of the Ministerio de Educación y Ciencia of Spain (Project AGL2006−01092).

JF070527W